REMARKS

Claims 13, 14, 19-22, 25, 26, and 31-35 are pending in the application. Claims 14, 19-22, 26, and 30 have been withdrawn from consideration as directed to non-elected inventions. Claims 32 and 34 have been canceled. Claims 13, 14, 19-22, 33 and 35 have been amended.

Entry of the Amendment

Entry of the amendment is respectfully requested. Claim 13 has been amended to recite that the administered amount of pravastatin or pharmacologically acceptable salts or esters thereof is "sufficient to enhance glucose uptake into warm-blooded animal cells." Applicants submit that the amendment clarifies the claimed invention, does not introduce new matter, does not raise issues that would require further consideration or search by the Examiner, and does place the application in condition for allowance. Independent Claims 14 and 19-22 have been similarly amended. Claims 33 and 35 have been amended to overcome the Section 112 rejection of Claims 32 and 34 and, like the amendment to Claim 13, these amendments do not introduce new matter and do not raise issues that would require further consideration or search by the Examiner. For these reasons, entry of the amendment is requested.

The Provisional Double Patenting Rejection

Claims 13, 25, 32, and 33 are provisionally rejected on the ground of non-statutory obviousness-type double patenting as then being unpatentable over Claims 41, 43-47, 57, 59, and 60 of co-pending Application No. 10/555,076. Applicants acknowledge the provisional rejection and will file a Terminal Disclaimer on an indication of allowed subject matter.

The Rejection of Claims 32 and 34 Under 35 U.S.C. § 112, First Paragraph

Claims 32 and 34 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Without acquiescing to the Examiner's rejection, Claims 32 and 34 have been canceled. Withdrawal of the rejection is respectfully requested.

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The Rejection of Claims 13 and 25 Under 35 U.S.C. § 102(b)

Claims 13 and 25 have been rejected under 35 U.S.C. § 102(b) as anticipated by Freeman et al., *Circulation* 103:357-372, January 2001 (the "Freeman reference"). Withdrawal of the rejection is requested for the following reasons.

Claim 13 is directed to a method for enhancing glucose uptake into warm-blooded animal cells. In the method, an effective amount of pravastatin or pharmacologically acceptable salts or esters thereof sufficient to enhance glucose uptake into warm-blooded animal cells is administered to a warm-blooded animal in need thereof. Claim 25 depends from Claim 13.

The Examiner states that the Freeman reference "clearly shows the administration of pravastatin beneficially affected glucose transport." The Examiner concludes that the activity of pravastatin produces glucose uptake unless the applicants can prove the statement "beneficially affect glucose and insulin transport" does not include or is not related to "glucose uptake."

Applicants believe that the statement "beneficially affect glucose and insulin transport" is not related to "glucose uptake" into a cell. Applicants offer the following remarks regarding the Freeman reference.

Taken in context, the sentence containing the quoted phrase reads "By restoring endothelial function, pravastatin may significantly influence selective tissue perfusion and thereby beneficially affect glucose and insulin transport." (Page 361, paragraph beginning with "Finally,".) The paragraph containing the quoted phrase makes clear that the term "endothelial function" is referring to physiological parameters such as vasodilatation, and how those parameters may affect transport of glucose and insulin across the endothelium into the target tissues (tissue perfusion), and not to enhanced uptake of glucose by cells in the tissues. Further, the Freeman reference does not describe any data showing that pravastatin influences transport of glucose into the tissues, or that improved transport of glucose into the tissues would result in

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enhanced uptake of glucose by cells. Therefore, the reference does not anticipate the method of Claim 13, a method for enhancing glucose uptake into warm-blooded animal cells by administering pravastatin.

Furthermore, the Freeman reference is not effective prior art under Section 102(b) because the reference does not enable one of skill in the art to practice the claimed method. See MPEP 2121.01 (The disclosure in an assertedly anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation. *Elan Pharm., Inc. v. Mayo Found. for Med. Educ. & Research*, 346 F.3d 1051, 1054, 68 USPQ2d 1373, 1376 (Fed. Cir. 2003)); See also *Novo Nordisk Pharmaceuticals, Inc. v. Bio-Technology General Corp.*, 76 USPQ2d 1811, 1816–17 (Fed. Cir. 2005) ("In order to anticipate, a prior art disclosure must also be enabling, such that one of ordinary skill in the art could practice the invention without undue experimentation."). The Freeman reference merely speculates that three known effects of pravastatin therapy may play a primary role, either individually or in concert in the development of diabetes. (Page 360, right hand column, second full paragraph.) The reference does not teach or suggest to one of ordinary skill how to practice a method for enhancing glucose uptake into warm-blooded animal cells. Rather, the reference teaches methods for determining an association between pravastatin therapy and a reduced risk of developing diabetes. As mentioned above, the Freeman reference fails to disclose methods for determining how pravastatin influences selective tissue perfusion and thereby transport of glucose into the tissues, or methods for determining how transport of glucose into the tissues results in enhanced uptake of glucose by cells. Therefore, the Freeman reference does not enable one of ordinary skill to practice the claimed invention without undue experimentation.

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Because the Freeman reference fails to exactly describe or enable one of skill in the art to practice the claimed invention, the reference is not anticipatory, and withdrawal of the rejection is respectfully requested.

The Rejection of Claims 31-35 Under 35 U.S.C. § 103(a)

Claims 31-35 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over the Freeman reference in view of U.S. Patent No. 5,643,868, issued to Weiner et al., and further in view of Paolisso et al., *European Journal of Clinical Pharmacology*, Vol. 40, No. 1, pp. 27-31 (1991). Withdrawal of the rejection is requested for the following reasons.

Claim 31 depends from Claim 13. Claim 31 relates to a method for enhancing glucose uptake into a warm-blooded animal cell comprising administering an effective amount of pravastatin or pharmacologically acceptable salts or esters thereof in the presence of insulin. Claims 32 and 34 have been canceled. Claims 33 and 35 depend from Claim 31.

The teaching of the Freeman reference is noted above in regard to the rejection of Claims 13 and 25. At most, the Freeman reference speculates that pravastatin may improve endothelial function and thereby affect glucose transport from the blood to the tissues. The Freeman reference does not teach or suggest that glucose transport into the tissues results in enhanced glucose uptake into cells. The deficiencies of the teaching of the Freeman reference noted above with regard to Claim 13 are not cured by the teachings of the Weiner et al. and Paolisso et al. references.

As acknowledged by the Examiner, the Freeman reference does not teach the administration of pravastatin in the presence of insulin, as recited in Claim 31, or the addition of a second HMG-CoA reductase inhibitor as recited in amended Claims 33 and 35. The Weiner reference is directed to methods for treating or preventing a disease in mammals having the characteristics of Type 1 diabetes, wherein the method includes the step of administering insulin

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or disease suppressive fragments of insulin or analogs to the mammals. The Paolisso reference discloses that administering simvastatin to non-insulin dependent diabetic patients improves the action of insulin as demonstrated by stronger inhibition of hepatic glucose output and stimulation of both the glucose disappearance rate and the glucose metabolic clearance rate.

The Weiner and Paolisso references do not teach the administration of pravastatin or pharmacologically acceptable salts or esters thereof for enhancing glucose uptake into warm-blooded animal cells. Therefore, the combined teachings of the cited references fail to teach every limitation of the claimed invention. The cited references simply fail to teach, suggest, or provide any motivation to arrive at the invention as now claimed: a method for enhancing glucose uptake that includes administering an effective amount of pravastatin or pharmacologically acceptable salts or esters thereof in the presence of insulin, and a method for enhancing glucose uptake that further comprises administering an effective amount of a second HMG-CoA reductase inhibitor.

Applicants offer the following remarks regarding the Weiner reference. The Examiner asserts that the Weiner reference teaches that the administration of insulin does not decrease blood sugar (glucose) in pancreatic beta cells in a mammal. However, applicants point out that the Weiner reference discloses that oral administration of insulin to mice did not result in a decrease in blood sugar glucose within 4 hours after administration of insulin. In contrast to the assertion of the Examiner, the Weiner reference does not disclose the effects of insulin on blood sugar in pancreatic beta cells, rather that oral administration of insulin reduces the number of inflammatory immune system cells surrounding the pancreas. (Column 8, lines 52-55).

Furthermore, when the prior art teaches away from the claimed invention, the invention is more likely to be non-obvious. *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1740 (2007). In the present case, applicants submit that the prior art teaches away from the claimed invention,

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and offer as evidence the non-patent publication to L.H. Chamberlain provided in **Exhibit A** ("Inhibition of isoprenoid biosynthesis causes insulin resistance in 3T3-L1 adipocytes," *FEBS Letters* 507:357-361 (2001)). Chamberlain discloses treating cells with lovastatin to inhibit isoprenoid synthesis in the presence of normal cholesterol levels. Chamberlain teaches that "2-deoxyglucose transport in response to insulin challenge was significantly reduced in cells treated with lovastatin, demonstrating that defects in isoprenoid metabolism can lead to cellular insulin resistance." (Page 360, left hand column, first paragraph.) Note also that lovastatin alone did not substantially increase glucose uptake into cells (see Figure 4). Therefore, as of the filing date, the prior art teaches that one of skill in the art would not have a reasonable expectation of success in enhancing glucose uptake into animal cells by administering a statin. Thus, the claimed invention produces the unexpected result that pravastatin enhances glucose uptake into warm-blooded animal cells, and would not have been obvious to one of skill in the art.

Because the cited references, either alone or in any combination, fail to teach, suggest, provide any motivation to make, or otherwise render obvious the claimed invention, the claimed invention is non-obvious and patentable over the cited references. Withdrawal of the rejection is respectfully requested.

Rejoinder of Withdrawn Claims 14, 19-22, and 26

Independent Claims 14 and 19-22 are currently amended to conform to currently amended Claim 13. Rejoinder and allowance of withdrawn Claims 14, 19-22, and 26 is requested.

CONCLUSION

In view of the above amendments and foregoing remarks, applicants believe that Claims 13, 14, 19-22, 25, 26, 31, 33, and 35 are in condition for allowance. If any issues remain

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that may be expeditiously addressed in a telephone interview, the Examiner is encouraged to telephone applicants' attorney at 206.695.1755.

Respectfully submitted,

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EXHIBIT A

FEBS 25400 FEBS Letters 507 (2001) 357–361

Inhibition of isoprenoid biosynthesis causes insulin resistance in 3T3-L1 adipocytes

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Abstract **Lovastatin treatment caused down-regulation of the insulin-responsive glucose transporter 4 (Glut4) and up-regulation of Glut1 in 3T3-L1 adipocytes. These changes in protein expression were associated with a marked inhibition of insulin-stimulated glucose transport. Lovastatin had no effect on cell cholesterol levels, but its effects were reversed by mevalonate, demonstrating that inhibition of isoprenoid biosynthesis causes insulin resistance in 3T3-L1 adipocytes. These findings support the notion that whole body insulin resistance may arise as a result of perturbations in general biochemical pathways, rather than primary defects in insulin signalling.** © **2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

Key words: Glut4; Glut1; Lovastatin; Cholesterol; Isoprenoid; Adipocyte

1. Introduction

Insulin stimulates the fusion of intracellular vesicles containing the glucose transporter 4 (Glut4) with the plasma membrane, resulting in an enhanced expression of Glut4 at the cell surface and an increased rate of glucose uptake [1,2]. Defects in the signalling and trafficking pathways that regulate Glut4 distribution and decreased Glut4 expression levels have all been implicated as contributing factors to insulin resistance and type 2 diabetes [3–6]. Whether defects in these insulin-regulated pathways are the primary cause of insulin resistance or are secondary to more general cellular abnormalities is unknown. It is therefore essential to identify metabolic changes that can cause an insulin resistant state.

A number of conditions have been identified that lead to decreased Glut4 expression and subsequent insulin resistance. These include chronic exposure to insulin [7,8], which decreases Glut4 mRNA levels and protein stability, and prolonged exposure to tumor necrosis factor α or arachidonic acid, which inhibit Glut4 transcription [9,10]. These decreases in Glut4 levels are often paralleled/followed by increases in Glut1 transcription. Recent work has shown that culturing of 3T3-L1 adipocytes in cholesterol-depleted media in the presence of an inhibitor of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase (to block cholesterol biosynthesis) significantly reduced cholesterol levels (three-fold), and resulted in down-regulation of Glut4 and up-regulation of Glut1 [11]. The changes in Glut4/Glut1 levels were evident at both mRNA and protein levels. Although cholesterol was clearly reduced in these cells, the synthesis of other isoprenoids (intermediates in the cholesterol biosynthetic pathway) would also be inhibited. Thus, it is not clear what impact inhibition of isoprenoid synthesis has on the expression of Glut4 and Glut1.

I have examined the effect of inhibiting isoprenoid synthesis (whilst maintaining normal cholesterol levels) on the expression of Glut4 and Glut1.

2. Materials and methods

2.1. Materials

Lovastatin was obtained from Calbiochem. Mevalonolactone, Infinity cholesterol reagent and cytochalasin B were from Sigma (Poole, UK). Glut1 antiserum was provided by Dr Steven Baldwin (University of Leeds, UK). Monoclonal insulin-responsive aminopeptidase (IRAP) antibody was from Drs Luis Garza and Morris Birnbaum (University of Pennsylvania, PA, USA).

2.2. Cell culture

3T3-L1 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum (FCS) at 37°C in a humidified atmosphere of 10% CO_2. Four days following confluence, the cells were incubated in DMEM with 10% FCS, 1 μg/ml insulin, 110 μg/ml isobutylmethylxanthinine, and 0.25 μM dexamethasone for 3 days, and then in FCS-containing media with 1 μg/ml insulin for a further 2 days. The cells were then incubated for 3–5 days in FCS media with or without lovastatin at the indicated concentrations.

2.3. Preparation of 3T3-L1 membranes

Cells were washed three times in HES (20 mM HEPES, 255 mM sucrose, 1 mM EDTA, pH 7.4) and homogenised by 20 strokes of a Dounce homogeniser in HES supplemented with a protease inhibitor cocktail (Boehringer Mannheim). Membranes were recovered by centrifugation at 196000×*g* for 1 h and resuspended in HES/protease inhibitors.

2.4. 2-Deoxyglucose transport

Adipocytes on 6-well plates were incubated in serum-free media for 2 h. The cells were then washed three times and incubated for 30 min at 37°C with or without 1 μM insulin. [^{3}H]2-Deoxyglucose (50 μM, 0.25 μCi) was added to each well for 3 min and the cells were quickly washed in ice-cold PBS, solubilised in Triton X-100 and [^{3}H]2-deoxyglucose uptake assayed by scintillation counting. Glut-specific glucose uptake was measured by subtracting values for [^{3}H]2-deoxyglucose uptake in the presence of 10 μM cytochalasin B.

2.5. Whole cell immunofluorescence

Adipocytes grown on collagen-coated coverslips were washed in PBS and fixed in methanol at −20°C for 6 min. The fixed cells

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Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; Glut, glucose transporter; IRAP, insulin-responsive aminopeptidase

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were washed in PBS and incubated for 10 min in PBS/1% foetal bovine serum (FBS). Antibodies against Glut4 and Glut1 (both at 1:100) in PBS/1% FBS were added to the cells for 1 h, and washed three times in PBS/5% goat serum. The cells were then incubated with secondary FITC-conjugated antibodies (1:200) in PBS/5% goat serum for 1 h, washed three times in PBS and processed for immunofluorescence analysis.

3. Results

To discriminate between effects of cholesterol and other isoprenoids on Glut4 and Glut1 expression, 3T3-L1 adipocytes were cultured in cholesterol-containing media with or without lovastatin. Lovastatin inhibits mevalonate formation by inhibiting the activity of HMG-CoA reductase, which catalyses the committed step in cholesterol biosynthesis. Treatment with 5 μM lovastatin for 3 days did not significantly affect membrane cholesterol levels as assayed using a cholesterol esterase/cholesterol oxidase colourimetric assay (ratio of cholesterol to protein was 0.070 ± 0.0036 for control cells and 0.071 ± 0.0048 for lovastatin-treated cells, $n = 6$). This implies that the major source of cholesterol is from uptake, rather than de novo synthesis. In contrast, incubation of 3T3-L1 adipocytes with 10 mM methyl-β-cyclodextrin for 30 min resulted in a 40% reduction in cellular cholesterol levels (not shown), confirming that the assay system can reliably detect cholesterol depletion. As an alternative measure of cholesterol levels, thin layer chromatography (TLC) analysis was performed on lyophilised cell pellets that had been subjected to an extended period of treatment with an increased concentration of lovastatin (10 μM for 7 days). TLC analysis revealed that even this prolonged treatment with lovastatin did not decrease cellular cholesterol levels (cholesterol levels in lovastatin-treated cells were 112 ± 5% that of control cells, $n = 3$).

The lack of effect of lovastatin on cholesterol levels is consistent with the work of Le Lay et al. [11] who showed that the effects of cholesterol depletion on SREBP-2 target genes was reversed when 1 μg/ml cholesterol was included in the growth media. This reversal occurred despite a sustained inhibition of cholesterol biosynthesis (by mevastatin), implying that cholesterol uptake and not de novo synthesis controls cellular cholesterol levels. Using this protocol, it is therefore possible to examine what impact inhibition of isoprenoid synthesis has on Glut4 and Glut1 expression.

Lovastatin treatment had a marked effect on both Glut4 and Glut1 expression levels (Fig. 1A,B), down-regulating Glut4 and up-regulating Glut1. These effects were maximal at 5 μM over 3 days of treatment. Glut4 levels were reduced to 43.2 ± 6.6% of control cells ($n = 6$, $P < 0.001$), whereas Glut1 expression was increased by 240 ± 31% ($n = 6$, $P < 0.01$). The effect of lovastatin on Glut4 and Glut1 expression was prevented when 250 μM mevalonate was also included in the growth media (Fig. 1A), strongly suggesting that the observed effects were due to an inhibition of HMG-CoA reductase and isoprenoid biosynthesis. In addition, expression of the Glut4 vesicle protein IRAP was reduced to 75.5 ± 6.6% ($n = 6$, $P < 0.01$) of control levels in lovastatin-treated cells.

Le Lay et al. [11] found that cholesterol depletion led to a 2.6-fold increase in caveolin 2 mRNA levels. In contrast, I found that lovastatin treatment produced a small but statistically significant decrease in caveolin 2 protein levels (79 ± 5.5% of control levels, $n = 6$, $P < 0.01$). This suggests

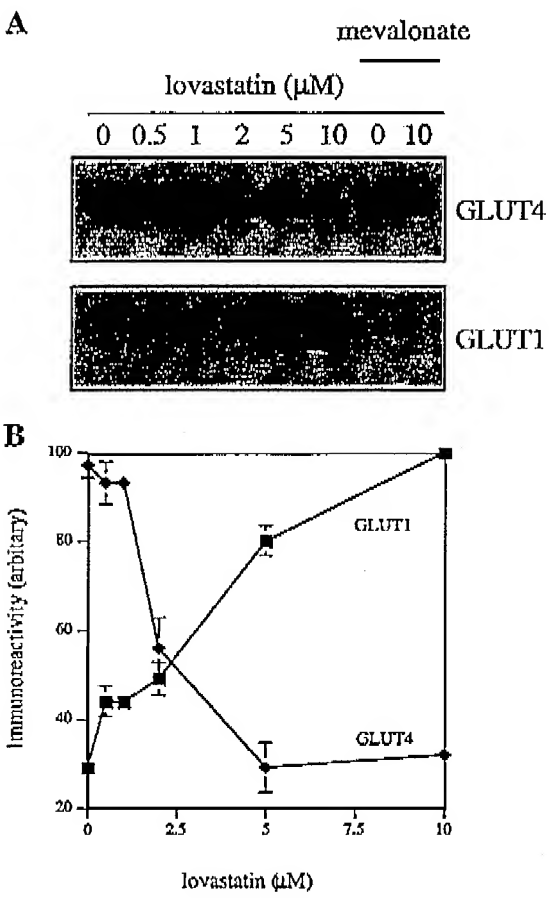

Fig. 1. Concentration dependence of lovastatin effects on protein expression. 3T3-L1 adipocytes at d5 post-differentiation were cultured in various concentrations of lovastatin (as indicated) for 72 h. 250 μM mevalonate was also added to the samples indicated. Cells were homogenised and membranes recovered by centrifugation. Equal amounts of the membranes were separated by SDS–PAGE and transferred to nitrocellulose for immunoblotting analysis using antibodies specific for Glut4 and Glut1. A: A representative immunoblot, whereas (B) is averaged data quantified from three separate experiments, expressed as means ± standard error.

that some of the changes in gene expression observed by Le Lay and colleagues were attributable to cholesterol depletion (caveolin 2), whereas others (Glut4 and Glut1) were caused by inhibiting the synthesis of cholesterol precursors.

To assess the time dependence of lovastatin effects on Glut4, Glut1 and IRAP expression, samples of adipocytes were analysed at various times following addition of lovastatin. 5 μM lovastatin was added to 3T3-L1 adipocytes on the fifth day following the initiation of differentiation, and protein levels analysed at subsequent 24 h intervals. Fig. 2A,B shows that lovastatin-induced increases in Glut1 expression were evident after 1 day of treatment, whereas Glut4 expression was significantly decreased after 2 days. In comparison, decreased expression of IRAP was evident after 3 days lovastatin treatment. The longer time (compared to Glut1) required to observe changes in Glut4 and IRAP expression are consistent with the idea that lovastatins effects are occurring at the

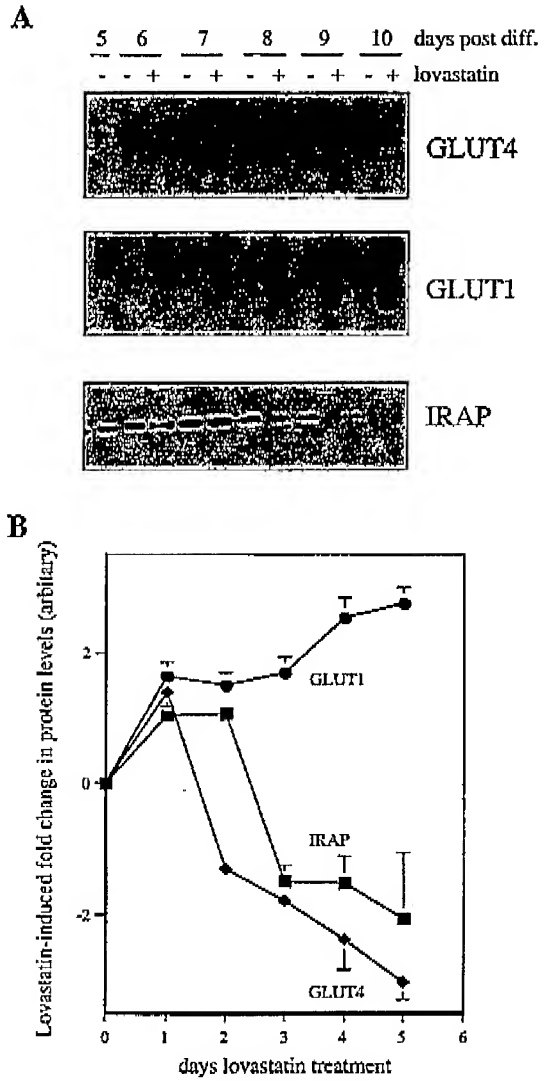

Fig. 2. Time dependence of lovastatin effects on protein expression. Lovastatin (5 μM) was added to 3T3-L1 adipocytes at day 5 post-differentiation, and membranes were prepared from the cells at subsequent 24 h intervals to day 10. Equal amounts of membrane proteins were separated by SDS–PAGE and transferred to nitrocellulose for immunoblotting analysis using antibodies against Glut4, Glut1 and IRAP. A: A representative immunoblot, whereas (B) is averaged data quantified from three separate experiments, expressed as means ± standard error.

mRNA level [11]. Note that lovastatin treatment resulted in the generation of a lower molecular weight Glut1 species (Figs. 1A and 2A), which may arise as a result of inefficient glycosylation (as discussed later).

To further analyse the effect of lovastatin treatment on cellular Glut4/Glut1 expression, adipocytes were examined by whole cell immunofluorescence. By this method, Glut4 levels also appeared to be diminished in lovastatin-treated cells (Fig. 3B) compared to control cells (Fig. 3A). Note that the laser power setting was three times greater for the image shown of lovastatin-treated, Glut4-stained cells than for control cells. In contrast, lovastatin-treated cells stained for Glut1 (Fig. 3D) appeared brighter than the corresponding control cells (Fig. 3C). Although the images shown do not provide quantitative evidence of changes in proteins expression, they do provide evidence that the changes in Glut4 and Glut1 expression are occurring throughout the cell population, rather than in a sub-population of cells. Fig. 3 also shows that lovastatin-treated 3T3-L1 adipocytes display a typical adipocyte morphology, with several fat droplets per cell.

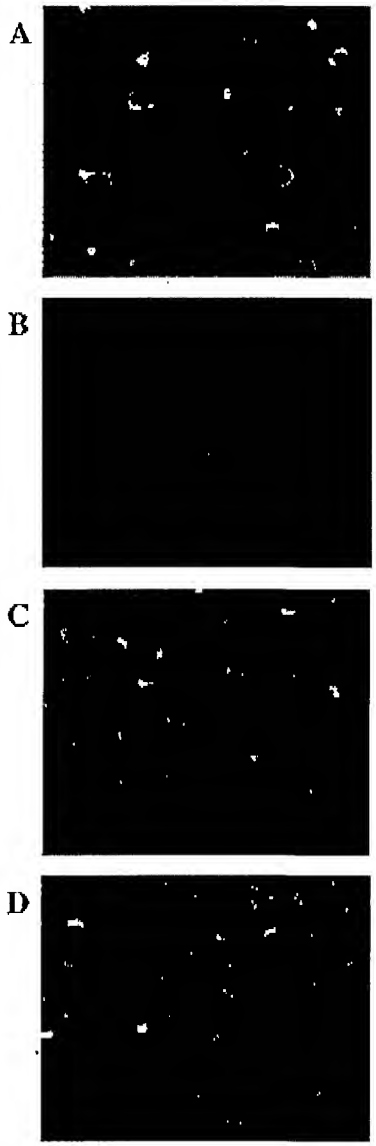

Fig. 3. Immunofluorescence analysis of Glut4 and Glut1 expression in lovastatin-treated 3T3-L1 adipocytes. Cells were incubated with or without 5 μM lovastatin for 3 days and processed for immunofluorescence analysis as detailed in Section 2. Shown are representative images of Glut4 in control (A) and lovastatin-treated cells (B), and Glut1 in control (C) and lovastatin-treated cells (D).

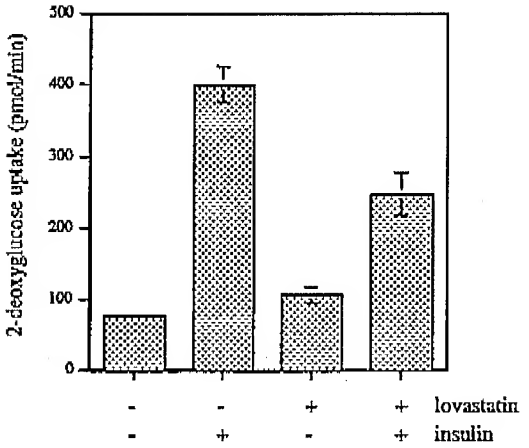

Fig. 4. Effect of lovastatin on insulin-stimulated glucose uptake into 3T3-L1 adipocytes. Cells were grown in the presence or absence of 5 μM lovastatin for 3 days. The cells were then incubated in serum-free media for 2 h, and [^{3}H]2-deoxyglucose uptake measured following treatment with (+) or without (–) 1 μM insulin for 20 min. The data are expressed as pmol glucose uptake/min/well ($n=3$).

To assess whether the decreased expression of Glut4 is associated with insulin resistance, insulin-stimulated 2-deoxyglucose transport into control and lovastatin-treated cells was assayed. Fig. 4 shows that 2-deoxyglucose transport in response to insulin challenge was significantly reduced in cells treated with lovastatin, demonstrating that defects in isoprenoid metabolism can lead to cellular insulin resistance.

4. Discussion

Numerous studies have examined the consequences on insulin action and whole body glucose homeostasis of mutating components of the insulin signalling cascade and glucose transport machinery [12–15]. Not surprisingly, in the majority of these cases insulin action is impaired. In contrast, the effects of perturbing general cellular pathways on insulin action has not been examined in any great detail. This study demonstrates that adipocytes become insulin resistant when isoprenoid biosynthesis is inhibited. In light of this, it is suggested that insulin resistance at the whole body level may arise as a result of imbalance in general biochemical pathways, and effects observed on insulin signalling pathways may occur secondary to this.

Previous work had shown that cholesterol depletion is associated with changes in Glut4 and Glut1 protein expression [11]. However, the protocol used in that study (combined cholesterol starvation and inhibited cholesterol biosynthesis) allowed the possibility that changes in gene expression were attributable to a decreased synthesis of isoprenoid cholesterol precursors, rather than to cholesterol itself. The procedure used in the current study lead to an inhibition of isoprenoid biosynthesis whilst maintaining normal cellular cholesterol levels (through an exogenous cholesterol source). Indeed, the lack of effect of lovastatin on cholesterol levels in 3T3-L1 adipocytes is consistent with a previous study [16]. Although cholesterol levels were normal, the synthesis of other isoprenoids were likely inhibited as mevalonate reversed the effects of lovastatin.

Possible mechanisms whereby lovastatin inhibits protein expression include an inhibition of protein prenylation (farnesol and geranylgeraniol are both intermediates in the cholesterol biosynthesis pathway). In this regard it is interesting to note that over-expression of the small GTPase ras in adipose tissue leads to an increased expression of Glut1 [17]. However, as lovastatin treatment would be predicted to inhibit ras activation, it is unlikely that the effects on Glut1 expression are linked to ras function. In addition, over-expression of ras was shown to have no effect on Glut4 expression [17].

Other points of note from this study are that the increased expression of Glut1 is unlikely to represent a compensatory mechanism to counter the decreased Glut4 expression. This is because increased expression of Glut1 is evident after 1 day of lovastatin treatment, whereas decreased Glut4 expression is observed following 2 days of treatment. Thus, it is likely that there is a coordinated (but opposite) regulatory mechanism controlling Glut4 and Glut1 expression. Interestingly, the observed decrease in IRAP expression followed the decrease in Glut4 expression. Although it is possible that this relates to different half-lives of the proteins, it may be that a decreased expression of Glut4 leads to a decrease in IRAP levels, as the two proteins appear to extensively colocalise and to traffic identically in adipocytes [18–21]. Finally, lovastatin treatment resulted in the synthesis of a lower molecular weight Glut1 species, which probably represents a non-glycosylated or partially glycosylated form of Glut1. This may arise because of lovastatin-mediated inhibition of dolichol biosynthesis (an important molecule for protein glycosylation) [22], alternatively, the increased expression of Glut1 may lead to inefficient glycosylation.

The results described in this paper are particularly relevant in light of very recent work showing that decreased expression of Glut4 in adipose tissue of transgenic mice causes insulin resistance in adipocytes, muscle and liver [15]. Thus, adipocyte-specific down-regulation of Glut4 may lead to pronounced whole body insulin resistance and ultimately type 2 diabetes.

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